A BIOLOGICAL SOIL TEST FOR AVAILABLE PHOSPHORUS BY SPONTANEOUS GROWTH OF SOIL ORGANISMS

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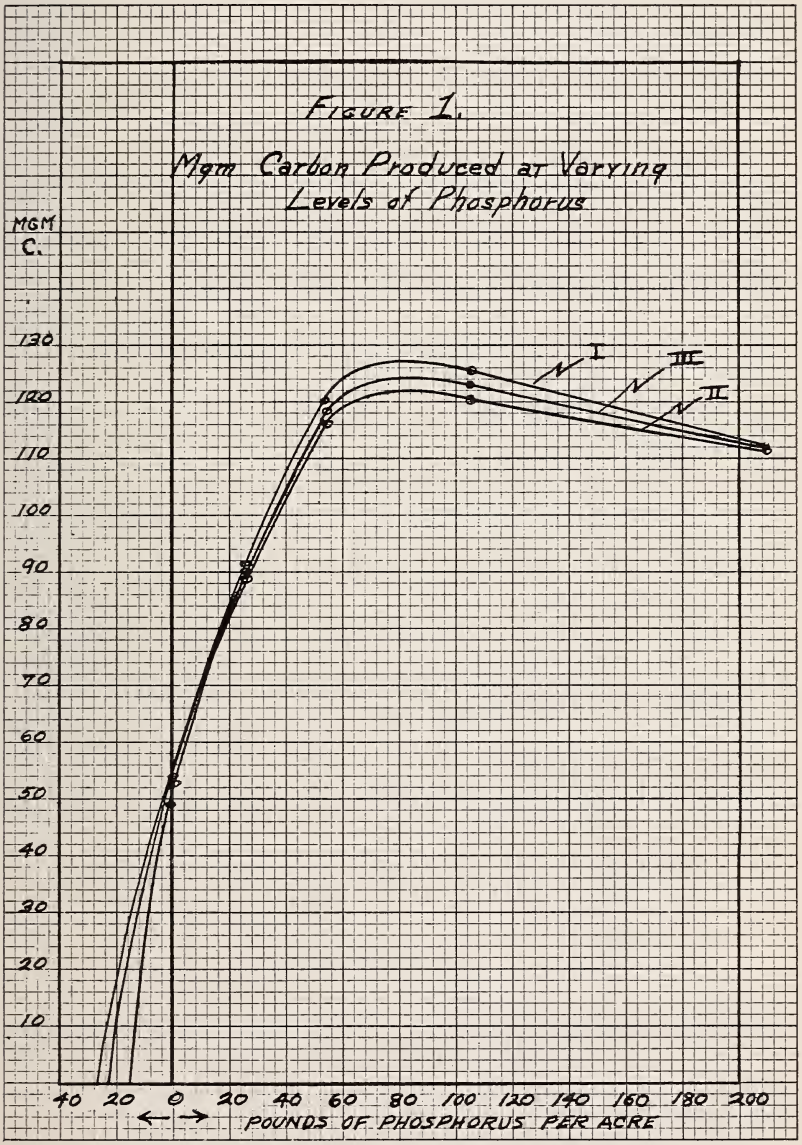
The search for a simple and more rapid biological technique to aid in determining available nutrients in the soil has been a desirable problem of long standing. Winogradsky (1925), using the *Azotobacter* soil-plaque technique, observed a close correlation between certain limiting factors for *Azotobacter* and those for growing plants. In 1927, he suggested that the responses of these microbes to calcium, phosphorus, and potassium could serve to indicate the limiting factors for these minerals with closer correlation to available amounts than may be achieved by chemical methods. Various ramifications of his basic technique followed (Sackett and Stewart, 1931; Halversen and Hodge, 1932). Results of these techniques as compared with Neubauer's seedling method (1923) showed both good and fair correlation (Stewart, *et al.*, 1931; Dahlberg and Brown, 1932; Green, 1932).

Another method which emanated from the earlier works of Butkewitsch (1909) in Russia was the *Aspergillus niger* technique. Through the efforts of Niklas, *et al.* (1930), and Niklas and Poschenreider (1936), there evolved a qualitative and quantitative method for the determination of available phosphorus, potassium, and to some extent, magnesium. Following this, Melich, *et al.* (1934), having modified Nicklas' method (1933), developed the *Cunninghamella*-plaque method for quantitative determinations of available phosphorus in soils. Comparisons between this latter method, the *Aspergillus niger* method, chemical methods, and field trials revealed good agreement with field test results.

It is to be noted that in all of the above cited references the techniques developed have utilized only isolated, specific cultures of soil organisms. However, those who are familiar with microbiology are well aware of the problems and the effort required to maintain aseptic techniques, as well as the storage of pure cultures. In the soil there exist not only the kinds of organisms used in these techniques mentioned, but countless numbers of other bacteria and fungi which are all classified as lower forms of plant life. They,

too, require phosphorus in varying amounts. The possibility exists that the combined numbers of spontaneous microbial growth in a relatively large sample of soil (one-half pound), when supplied with varying quantities of phosphorus (other nutrients being constant),



would increase in growth with a ratio commensurate with the amounts of phosphorus available. As these numbers increase, their rate of respiration (carbon dioxide) would also increase proportionately. By measuring the amounts of CO_2 evolved at varying rates of phosphorus supplied, an indication of the phosphorus available to these organisms could be ascertained. It was for this purpose that the following project was undertaken.

Method

Since the method presented is designed to be preliminary in nature, a soil type that was known to fix phosphorus in relatively large amounts was selected. A Red Bay sandy loam, taken from a corn field near Marianna, Florida, was utilized. The soil was air-dried and sieved through a 20-mesh screen. One-half pound quantities of soil were weighed and placed in the required number of clean two quart glass topped mason jars.

The nutrient level used was based on an application of 1,000 pounds of a 6-6-6 fertilizer per acre. Amounts of phosphorus were made to vary in each set of three replications from zero percentage P_2O_5 to 48 per cent, by increments of none, 6, 12, 24, and 48 per cent P_2O_5. Calcium and magnesium as dolomitic limestone, and the minor elements in the form of "FTE" [1] were also added. These were applied at rates of 2,000 pounds and 200 pounds per acre respectively. As a rapid source of energy, sucrose, based on 1 gram per one-half pound of soil, was included. Sources of nitrogen, phosphorus, and potassium were added as the salts, NH_4NO_3, Na_2HPO_4, and KCl in solution form. By using measured amounts of these solutions, accuracy and uniformity of the experiment was increased. The following quantities were dissolved separately in one liter amounts of distilled water:

Nitrogen	1.94 gms. of	NH_4NO_3
Phosphorus	1.36 gms. of	Na_2HPO_4
Potassium	1.07 gms. of	KCl
Carbon	500.00 gms. of	Sucrose

One ml. of each solution, except sucrose, yields the equivalent of 6 per cent nitrogen, P_2O_5 and K_2O respectively. Thus, volumes zero, 1, 2, 4, and 8 ml. of the Na_2HPO_4 solution corresponded to the

[1] Fritted Trace Elements, Ferro Co. (Dupont product.)

desired variations of phosphorus. All quantities were calculated on the basis of 2,000,000 pounds per acre of mineral soil, 6-inch depth. As a source of energy, the sugar solution was added at the rate of 1 gm. per half-pound sample of soil, or the equivalent of 2 ml. of sugar solution. Dolomite and FTE powder were mixed, weighed, and incorporated in the soil. Since the volume of nutrient and energy solutions that were added to the soil were not sufficient to moisten the entire volume, it was decided to augment each portion of soil in the jars by a quantity of water, until all soils had the equivalent of 20 ml. of solution. The amount of water added was sufficient to bring the soil to about 40 per cent of saturation.

After the solutions had been added, the soil were thoroughly mixed to insure uniformity. The uncapped jars were placed in an incubator which had previously been set to maintain a temperature of 28 degrees C. These jars were allowed to remain uncapped for a period of 12 hours. Following this, 50 ml. quantities of 0.5 N. NaOH were placed in small, uniform size, dispensary bottles. By means of a fine wire secured around the neck of the bottle, they were lowered into the glass jars until their bases rested on the soil suface. The opposite end of the wire was allowed to hang over the rim of the jar. The jars were sealed and allowed to incubate for 31 hours at 28 degrees C. Temperature is critical and should be maintained at a uniform condition in all parts of the incubator.

At the termination of this period, the jars were removed from the incubator, unsealed, and the dispensary bottles removed. Each 50 ml. quantity was poured into a 125 ml. Ehrlenmeyer flask. To precipitate the carbon as the carbonate, an excess of 2 N. $BaCl_2$ was added. With phenolphthalein as an indicator, the remaining NaOH was titrated with 0.5 N. HCl. To determine the amounts of carbon produced,[2] the following formula was used:

Ml. of NaOH used—ml. standard HCl used x 3 = mgm. of carbon.

Results

After titrating the remaining NaOH against the standard acid and employing the formula, the values expressed as mgm. of carbon for the three replications are shown in Table 1.

[2] Amounts may be expressed in mgm. CO_2 evolved by substituting the factor 11 for 3.

TABLE 1

Mgm. of Carbon Produced at Varying Levels of Phosphorus

Percent P_2O_5 (Fertilizer Formula)	Lbs./Acre (Phosphorus Applied)	Replications		
		I	II	III
0	0.0	49.5	54.0	52.8
6	26.16	91.5	88.8	89.7
12	52.32	120.0	116.4	118.2
24	104.64	125.4	119.7	123.0
48	209.28	111.9	111.3	111.9

The mgm. of carbon plotted against the quantities of phosphorus, in terms of pounds per acre added, are shown on the graph (Figure 1). A smooth curve is constructed for each replica and extended to the base-line to determine the approximate pounds of available phosphorus per acre. For this particular sample of soil, the available phosphorus, as indicated by the graph, is between 15 to 27 pounds per acre.

Discussion and Summary

The similarity of the three curves suggests a correlation between the amount of phosphorus available to the organisms and the amounts of CO_2 evolved, or the amount of carbon produced. Leaving the jars uncapped during the latent or initial stationary phase enables the microorganisms to adjust to the new environment and enter a phase of accelerated growth. All other factors being constant during this and the phase of logarithmic increase, phosphorus would serve as the limiting factor.

Preliminary experiments indicated that incubation in excess of 60 hours created a noticeable negative oxygen tension which may be assumed to adversely affect normal microbial growth and phosphorus utilization. All indications showed normal growth and uniform CO_2 evolution for the 30 hours incubation period. The break at the peak of the curves were assumed to be the effects of excessive phosphorus.

The slope of the smooth curves at the lower values for phosphorus strongly suggests the possibility that an extension of these lines to the base of the graph enables the investigator to determine the initial quantity of phosphorus available in the untreated sample

of soil. By fundamental geometric axioms, it becomes evident that the point of interception of the slope of the line with the abscissa, whether to the left or to the right of the ordinate, is basically the same. Therefore, in this case, the range of available phosphorus can be estimated to be between 15 and 27 pounds per acre for the untreated soil sample.

A new method, utilizing spontaneous microbial growth as a rapid, practical biological method for determining the approximate quantities of available phosphorus is offered. Microbial activity is shown to increase in proportion to the amounts of available phosphorus supplied. Future possibilities are suggested by correlation with field trials for establishing the amounts of available phosphorus in soils.

Literature Cited

BUTKEWITSCH, W.

1909. Die Kultur des Schimmelspilzes *Aspergillus Niger* als Mittel zur Bodenuntersuchung. *Zhur. Opytn. Agron.* (Russian Jour. Landw.) 10: 136-141.

DALBERG, H. W., and R. J. BROWN

1932. A Study of the Neubauer and Winogradsky (Azotobacter) Methods as Compared with a Chemical Method for the Determination of Phosphate Deficiency in Western Soils. *Jour. Am. Soc. Agron.* 24: 460-468.

GREEN, R. A.

1932. The Applicability of the Azotobacter (Plaque) Method for Determining the Fertility Requirements of Arizona Soils. *Soil Sci.* 34: 83-94.

HALBERSEN, W. V., and W. G. HOGE

1942. The Azotobacter Plaque Test as Applied to the Determination of Phosphate Deficiency in Idaho Soils. *Jour. Am. Soc. Agron.* 34: 503-512.

MEHLICH, A., E. B. FRED, and E. TRUOG

1934. The *Cunninghamella* Plaque Method of Measuring Available Phosphorus in Soil. *Soil Sci.* 38: 445-461.

NEUBAUER, H., and W. SCHNEIDER

1923. Die Nahrstoffaufnahme des Keimpflanzen und ihre Anwendug suf die Bestimmung des Nahrstoffgehalts de Boden. *Ztschr. Pflanzenernahr. u. Dungung.* (a) 2: 329-362.

NIKLAS, H., and H. POSCHENRIEDER

1932. Die Ausfuhrung der *Aspergillus* Methode zur Prufung auf Kali. *Ernahr. Pflanze.* 28: 86-88.

1936. Zur Feststellung der Magnesia-Dunge-Bederetigkeit und Magnesia-Dungewirkung Im Boden Mittels *Aspergillus* Niger. *Bodenk. u. Pflanzenernahr.* 1: 235-247.

NIKLAS, H., H. POSCHENRIEDER, and J. TRISCHLER

1930a. Eine N eue Microbiologische Methode Zur Feststellung der Dungebedurftigkeit der Boden. *Ztschr. Pflanzenernahr., Dungung, u. Bodenk.* (a) 18: 129-157.

1930b. Die Kultur des Schimmelspilzes *Aspergillus* niger zur Biochemischen Bestimmung de Kali und Phosphorsaurebedurftigkeit der Boden. *Ernahr. Pflanze.* 26: 97-103.

SACKETT, W. G., and L. C. STEWART

1931. A Bacterial Method for Determining Mineral Soil Deficiencies by Use of the Soil Plaque. *Colo. Agri. Exp. Sta. Bul.* 375: 1-36.

STEWART, L. C., W. G. SACKETT, D. W. ROBERTSON, and A. KEZER

1932. A Comparison of the Soil Plaque Method with the Neubauer and Hoffer Corn Stalk Methods for Determining Mineral Soil Deficiencies. *Colo. Agri. Exp. Sta. Bul.* 390: 1-59.

WINOGRADSKY, S.

1925. Etudes sur la Microbiologie du Sol: II. Sur les Microbes Fixateurs d'Azote. *Ann. Inst. Pasteur* 40: 455-520.

1927. Etudes sur la Microbiologie du Sol: III, Sur Le Pouvoir Fixateur les Terres. *Ann. Inst. Pasteur* 42: 36-62.